MORTALITY IN PIKE-PERCH EGGS IN HATCHERIES.[1]

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The remarkable losses in the hatching of pike-perch (*Stizostedion vitreum*) eggs have frequently made this phase of fish culture a ground for investigation. It is, however, not unjust to say that very little detailed study has been made and that no definite conclusion as to the causes of the high death rate has ever been reached.

The methods used in the handling of parent fish and eggs are, in the main, very much alike at the various stations that hatch pike perch. The fishes are caught in nets that are usually pulled once a day, weather permitting. Those that are ripe are stripped immediately, either in the field or at the hatchery, while the rest are retained in pens or live boxes. They are examined in turn daily until found to be ripe. In some years the sexes are found to be disproportionate in number. If males are scarce, the same individual may be used to obtain milt on several successive days. Fertilization is by the dry method, no water, or very little water, being used in the process. Milt and eggs are stripped into a bowl in more or less regular alternation, and the whole is gently stirred at frequent intervals to insure the contact of the eggs with the sperm. When the bowl is sufficiently full—generally after 10 to 15 minutes—the contents are diluted with water which after a varying period is poured off and renewed until the eggs are contained in clear water. Cohesion of the eggs, which at this time are extremely sticky, is prevented by active stirring or by adding silt or starch to the water in addition to such mechanical agitation. Finally, after several hours, the eggs are put into the hatching jars through which a gentle current of water is kept flowing.

As already indicated, the losses are very great. Nevin (1887) considers a hatch of 50 per cent a very fair success, and this would be agreed to by most fish-culturists. The cause of this great mortality is, in general, ascribed to failure of the eggs to be fertilized or else to injuries incurred while the eggs are being handled, especially the active stirring and the addition of foreign materials to prevent cohesion. It seems almost certain that these last-named crude procedures—which so far are unavoidable—are very apt to be

[1] Appendix V to the Report of the U. S. Commissioner of Fisheries for 1922. B. F. Doc. No. 926.

harmful, and a certain amount of loss is probably due to them. Reighard (1890) found that by very careful handling of the eggs at and immediately after fertilization the percentage of eggs that started segmentation soon after fertilization could be considerably increased. Describing the loss met with in the ordinary course of the routine methods, he states that in 252 samples examined 11 per cent had died due to lack of impregnation and 33 per cent due to injury. Unfortunately, although he designates the day and hour at which these observations were made, the age of the eggs is not specifically given. From the context it would appear as 29 hours. It seems that an egg was designated as dead when it showed an opaque white color, a criterion which was adopted also in the present investigation.

The figures of the losses in ordinary handling of pike-perch eggs given by Reighard are somewhat at variance with those given by L. H. Almy in some unpublished notes on the pike perch. His findings and those of the authors follow:

Almy.		Schrader and Schrader	
Age of eggs.	Per cent loss.	Age of eggs.	Per cent loss.
3 hours	2.5	1 hour	0.5
27 to 29 hours	5.4	4 hours 30 minutes	1.0
2 days	8.1	8 hours 15 minutes	2.4
3 days	12.6	19 hours	4.3
4 days	31.7	29 hours	5.0
5 days	35.5	2 days	8.3
7 days	39.0	3 days	13.3
9 days	33.4	4 days	33.2
		5 days	37.1

It will be seen that in contrast to the 33 per cent of white eggs given by Reighard, Almy observed only 5.4 per cent at 27 hours, while our own observations are lower still. Almy's and our figures agree fairly well, the latter being lower up to two days and a trifle higher at four days. It is not quite clear to what such a discrepancy could be due, although Reighard's hypothesis of injury as a cause of mortality would, of course, itself allow for large differences on account of the varying skill and care bestowed on the eggs. (Temperature conditions were in all cases apparently the same, the water being in the neighborhood of 45° F.) Reighard describes the injury as taking place most easily over the oil globule, and there is no reason to dispute the observation. However, the following explanation which he advances to account for this phenomenon does not seem to rest on a very firm physical basis (Reighard, 1890, pp. 33, 34):

> In the natural position the yolk sphere lies with its lower half against the egg membranes. These membranes, therefore, support this half of the yolk, surrounding it as if it were resting at the bottom of a cup. The upper half of the yolk is, on the contrary, not of the same form as the investing membrane; its spherical surface is interrupted by the protruding oil globule.
>
> The result of this arrangement is that when any pressure is brought to bear on the egg membranes, so that the space within which the yolk lies is reduced, the yolk is able to resist this pressure by fitting itself against the egg membrane at every part of its surface except over the oil globule. The strain,

therefore, comes on that part of the protoplasmic investment of the yoke which covers the oil globule and here it bursts. In almost every case the white spot which indicates the rupture of the yoke investment makes its appearance at the oil globule, usually at the equator.

Almy's as well as our own observations show that the death rate increases rapidly and steadily to the fourth day and then advances more slowly. To begin with, it must be noticed that a small percentage of dead eggs is found practically as soon as the fishes are

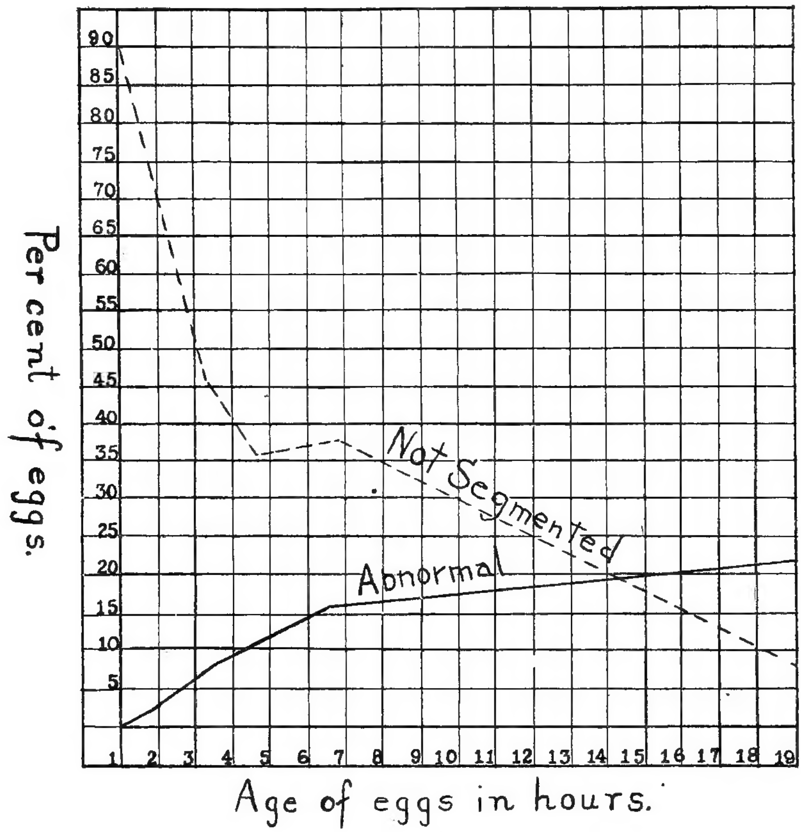
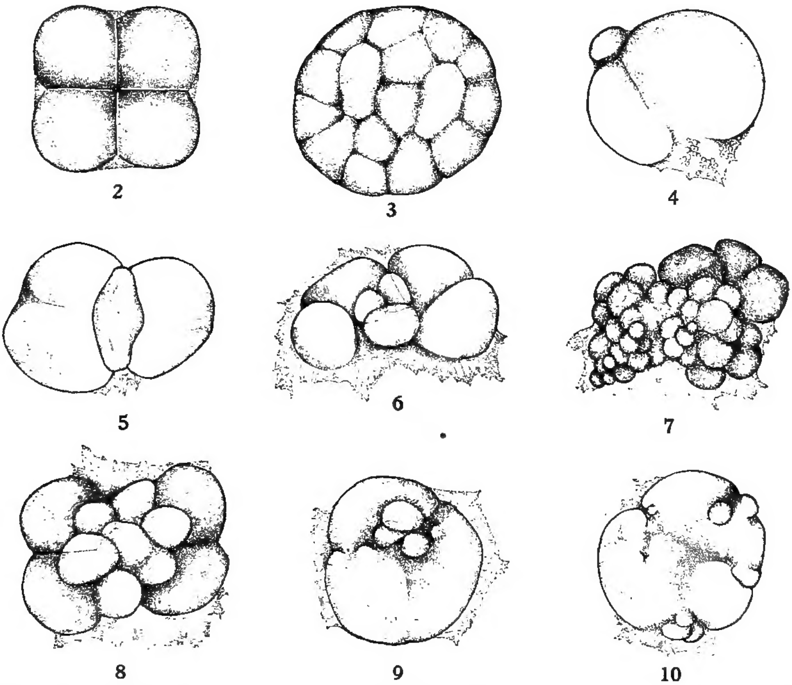


FIG. 1.—Graph showing the variation in numbers of abnormal and unsegmented pike-perch eggs during development.

stripped. It is surmised that these may have been injured in the process of stripping, or that they may have died through some developmental irregularity while still in the fish.

Coming now to the hypothesis that failure of fertilization is responsible for a greater part of the mortality of hatching eggs, it is generally assumed that lack of impregnation and failure to segment are closely correlated. A detailed examination of the material does not bear this out. We found that in eggs 4 hours 30 minutes old there was a considerable percentage which showed no trace of cleavage. This was true also at 5 hours 30 minutes, 6 hours 45 minutes, and

even 8 hours 15 minutes. Most of the unsegmented eggs at the latter stages on being examined cytologically appeared to be normal. They were therefore merely lagging behind. The proportion of eggs which has failed to cleave becomes progressively less with age, which in itself supports the idea that we are dealing here rather with a delay in cleavage than with a lack of impregnation. The curve in figure 1 (p. 3) shows graphically the numerical conditions encountered. These data are emphasized here merely to show that it is manifestly impossible even after eight hours to designate eggs as unfertilized when the absence of segmentation is taken as a criterion.



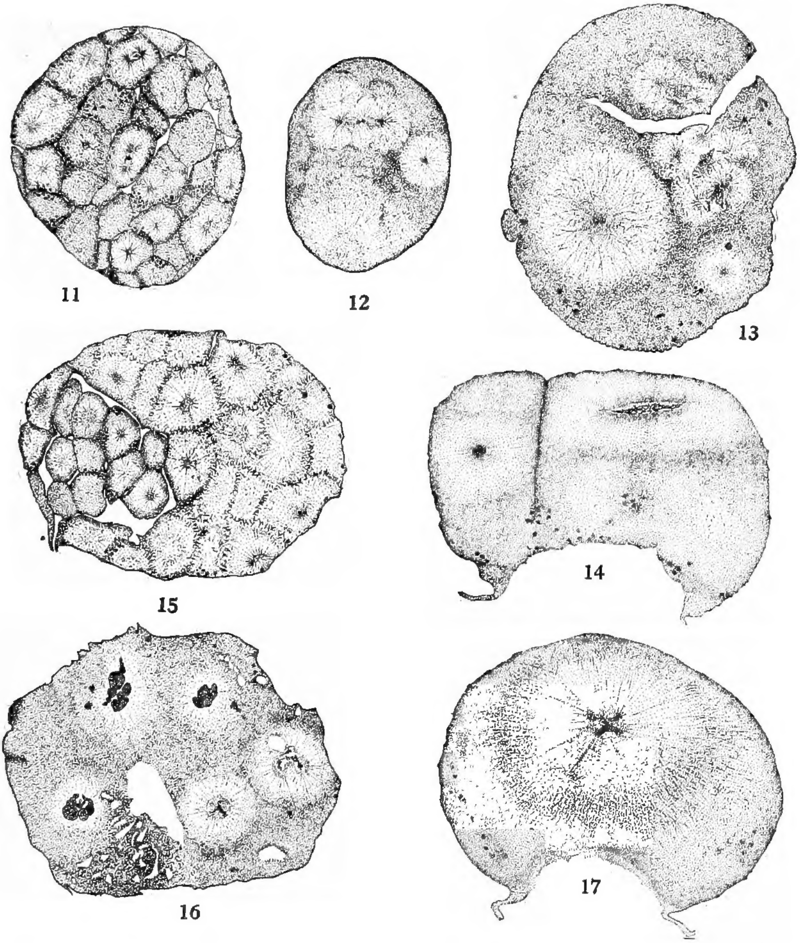
FIGS. 2 to 10.—Surface views of pike-perch eggs. Magnification, approximately × 100.

FIG. 2.—Normal 6-hour 4-celled blastoderm.
FIG. 3.—Normal 19-hour blastoderm.
FIG. 4.—Abnormal 8-hour blastoderm.
FIG. 5.—Abnormal 10-hour blastoderm.
FIG. 6.—Abnormal 10-hour blastoderm.
FIG. 7.—Abnormal 19-hour blastoderm.
FIG. 8.—Abnormal 19-hour blastoderm.
FIG. 9.—Abnormal 29-hour blastoderm.
FIG. 10.—Abnormal 29-hour blastoderm.

In addition to all this it must be considered that in the artificial insemination of the pike perch the eggs are immersed in milt which is diluted very little. The chance of a normal ripe egg remaining unfertilized must therefore be extremely small, and, as a matter of fact, it is surprising that polyspermy is not more often encountered.

In addition to the eggs which are found to be dead almost immediately, and to those which are slow to cleave, there is a third class which has been designated as "abnormal." In explanation it must be stated that minor irregularities in cleavage are not necessarily an indication of pathological conditions (H. V. Wilson, 1891), and only such extreme cases as are shown in figures 4 to 10 were rated as

abnormal. Eggs showing normal cleavage are shown in figures 2 and 3. Extreme variations, such as are shown in figures 4 to 10, in size of cleavage cells were found in nearly all cases to be correlated with internal conditions which presaged embryonic death sooner or



FIGS. 11 to 17.—Sections of pike-perch eggs. Magnification, approximately × 200.

FIG. 11.—Horizontal section of a normal egg of 64 or more cells.
FIG. 12.—From a 29-hour egg showing cytasters and abnormal spindles.
FIG. 13.—From a 29-hour egg showing size variation in cytasters.
FIG. 14.—From an 8-hour 15-minute egg, showing elongated nucleus.
FIG. 15.—From a 29-hour 15-minute egg, showing partial segmentation.
FIG. 16—From a 29-hour 15-minute egg showing degeneration in chromatin and multiplication of chromosomes.
FIG. 17.—From an 8-hour 15-minute egg showing monaster.

later. The number of such abnormal eggs increased steadily with age, ranging from 1 per cent at 4 hours 30 minutes to 21 per cent at 19 hours. As the curve in figure 1 shows, this increase runs parallel with a decrease in the number of unsegmented eggs, which suggests the possibility that such abnormal cases are derived chiefly

from eggs which are slow to cleave. It may be remarked that it is difficult to draw a strict line at times between the abnormal and the

a

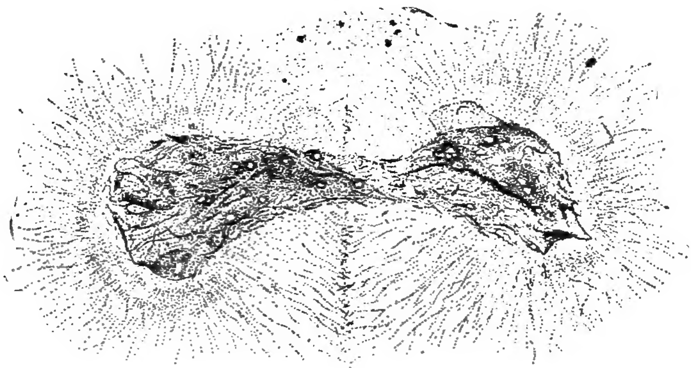
b 18

c

19

20

21



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Figs. 18 to 22.—Sections of pike-perch eggs. Magnification, approximately × 1620.

Fig. 18*a*, 18*b*, 18*c*.—Side views of three sections of a plate showing 30 chromosomes, the normal number.
Fig. 19.—Side view of a plate in another cell in the same blastoderm as figure 11, showing 15 chromosomes, the haploid number.
Fig. 20.—Polar view of normal plate.
Fig. 21.—Abnormal multiplication of chromosomes.
Fig. 22.—Abnormal elongated nucleus—abortive division.

unsegmented types in the first few hours. At that time one or a few minute excrescences are occasionally budded from the germinal disk,

and it becomes a question whether these should be regarded as cells or not.[2] On the other hand, at the 29-hour stage, abnormal eggs are often segmented into such small cells in such a way that, externally, it is very difficult to tell them from normal eggs, although internally they may be shown to be very irregular in behavior. The intermediate stages are therefore the best material for this phase of the investigation.

Those cytological features in the development of normal eggs which bear on the work in hand are as follows: Up to the 16-cell stage cell walls are sometimes partially or completely absent, but asters and spindles are normal in size and occupy the same position that they would if there were a distinct separation into cells. Following the 16-cell stage, the cleavage, which becomes externally complete at least in the surface layer of cells, gives evidence of this fact internally by the presence of very distinct cell walls. Mitosis is at first synchronous in all the cells, but this regularity is soon lost, so that certain cells of an egg may be in the resting condition while neighboring cells may be undergoing mitotic division.

As already indicated, cytological examination of uncleaved eggs at 4 to 8 hours showed the majority to be normal (fig. 11, p. 5). The few exceptions were found to have anomalous mitotic figures, and their number was increased in the 8-hour stage. At 29 hours every uncleaved egg showed anomalous internal features. The exceptional 8-hour eggs often show a very large monaster (fig. 17, p. 5). Other eggs may show several cytasters and an occasional spindle (figs. 12 to 16, p. 5). At 29 hours no such large monasters are found in eggs of this type or in those called abnormal and generally there is only an increase of cytasters in the former.

The abnormal eggs often present a curious mixture of spindles and asters of varying sizes, drawn-out nuclei, chromosomal irregularities, and partially formed cell walls (figs. 12 to 17, 21, and 22). Frequently an egg is found in which a part has undergone regular cleavage while the rest is filled with cytasters and shows no indication of cell walls (fig. 15). As it was expected that such irregularities would be reflected in the distribution of the chromosomes in division, evidence of such chromosomal abnormalities was sought. But, as in other teleosts, the chromosomes are usually so clumped that an exact analysis of them is very difficult. In at least one case, however, the metaphase plate in one cell showed close to 30 chromosomes (which seems to be the diploid number as obtained from counts in normal eggs, fig. 20), while the adjoining cell contained only about 15 (figs. 18 and 19). This might be explained as a case of partial fertilization, in which the sperm has instigated a division of the egg nucleus and later has fused with one of the nuclei resulting from this first division of the egg nucleus. The fusion nucleus would then be diploid and the purely maternal nucleus haploid.

Irregularities in cleavage and mitotic figures practically identical with those here described have been obtained experimentally by a number of investigators. It will be noted that in every experiment of this nature the effect is to induce development with one of the

[2] Reighard (1890a) mentions excrescences as occurring in correlation with the flow of protoplasm in the formation of the protoplasmic cap. Since the number of eggs showing the excrescences mentioned above increases long after the formation of the cap, in our case, the phenomenon described by Reighard is probably not related to it.

parent nuclei absent or in a weakened condition, with both in a weakened condition, or with the pronuclei incompatible through hybridization. A limited survey of the extensive literature on this subject will suffice to show the trend of the work.

E. B. Wilson (1901) found that in artificial parthenogenesis, where, of course, only one of the parent nuclei is present, there occur such abnormalities as the formation of cytasters, the multiplication of chromosomes without accompanying cell division, multipolar mitoses, and delay in cleavage. The chromosome number in the eggs which seem to show normal development is haploid.

Dungay (1913) weakened or injured sperms of several species of invertebrates by means of chemical treatment, heat, or staling, and development in eggs fertilized by such sperms resulted in delayed cleavage, abnormally sized cells, multipolar figures, and similar defects.

O. Hertwig (1911) and G. Hertwig (1912), among others, have described the effect of fertilizing eggs with sperms treated with radium. Both authors remarked especially a budding phenomenon correlated with delayed development. They also describe drawn-out nuclei, multiplication of chromosomes in a nucleus, giant nuclei, and cytasters, all almost identical with phenomena which we have described in the abnormal pike-perch eggs.

C. Packard (1914) found that sperms which had been treated with radium may stimulate the eggs to cleave but fail to take part themselves in the subsequent development. When the eggs are "radiated," they show various irregularities, such as abnormal divisions or the failure of pronuclei to unite.

G. and P. Hertwig (1914) produced similar effects to those already mentioned by weakening sperms with methlyn blue among other reagents. Still more striking are the phenomena produced by fertilizing the eggs of teleosts with sperms of another species of teleost. The whole list of abnormalities given above was reproduced in such development.

It is not within the province of applied biology to go into a theoretical consideration of these phenomena. Suffice it to say that the weakening or injury of either sperms or unfertilized eggs will produce the same defects in the development of all animals so far investigated. Physiological and cytological phenomena identical in appearance with those produced experimentally in this way have been observed also in abnormally developing pike-perch eggs, and it suggests itself that the cause of such irregularities is of similar nature. In other words, there is a weakening of either sperms or eggs before fertilization.

It is highly improbable that natural conditions should induce a state that would cause such a large mortality in the embryos, and it becomes almost certain that the injury is incurred during the period of the captivity of the fishes.

As has been said in the introduction, most of the fishes are found not to be ready for stripping when first caught. They are therefore retained in pens or crates until the reproductive products can be obtained from them by stripping; in other words, until they are "ripe." The penning of fishes prior to spawning is a practice of

long standing in the handling of pike perch as well as many other species. Some of these species are known to stand such confinement fairly well, but many show various ill effects, such as hardening of the ovaries, wateriness of milt, and low percentage of hatched fry. Whatever the cause and physiological process involved, be it abnormal hydrogen ion concentration due to the crowding of the parent fishes, or more directly circulatory and nervous relations, the result is a degeneration of eggs and sperms. In the case of the pike perch especially the consequent mortality may, of course, fluctuate from year to year due to such causes as sudden changes of temperature (a sudden change of temperature is known to materially retard the ripening of the fishes in the pens) and weather conditions which may prevent pulling the nets and therefore postpone examination of the caught fishes. Some specimens do not lay eggs even when ripe under such conditions.

It may be of interest in this connection to give the opinions of men who have the supervision of pike-perch hatcheries which are located at Constantia, N. Y., Swanton, Vt., Put in Bay, Ohio, and Duluth, Minn. Their opinions, given in response to a letter of inquiry, are

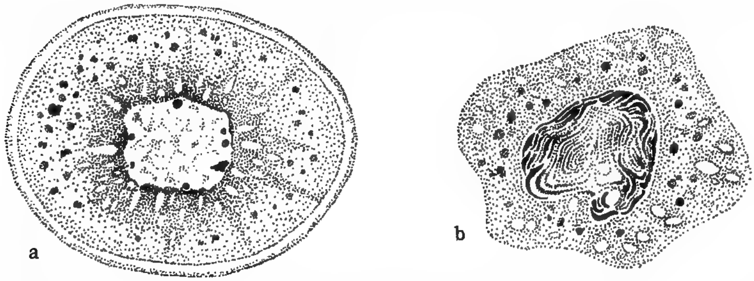

FIG. 23.—Eggs of Stenotomus prior to maturity. *a*, Normal egg; *b*, egg from a parent kept in a tank for two weeks.

not based on numerical data but are the results of practical observation. All of these four superintendents and a fifth, who was formerly connected with pike-perch work, agree that the mortality of eggs is proportional to the time that the adult pike perch are retained in pens and, conversely, that the percentage of hatched eggs from fish stripped when taken from the net is much greater than that of eggs from penned fish. Four of the men believe that both male and female are affected by penning, but that the female is more susceptible, while the fifth does not commit himself on this point but cautions against using the males more than once, i. e., on several days.

Bearing more directly on the problem are some experiments made by the senior author in connection with some other work. Females of the common scuppaug (*Stenotomus chrysops*) were netted shortly before the spawning period and retained in a tank supplied with a continual flow of fresh sea water. Specimen of these impenned fishes were dissected at intervals of a few days and the ovaries examined histologically. A progressive deterioration of the nearly ripe ova was observed, which at the end of two weeks had reached such a stage as shown in figure 23.

The generalization that the high death rate in pike-perch eggs is due to lack of impregnation thus seems to be unwarranted. That the present methods of preventing cohesion of the eggs are responsible for a certain percentage of the mortality is probable, but they do not account for all the loss. On the other hand, it has been shown that about 25 to 30 per cent of representative samples of 29-hour eggs show abnormalities that must lead to either malformation or death. If an average loss is then considered as 50 to 60 per cent (and that is a fair estimate), about half of this is due to the agency which manifests itself in abnormal development. This cause is in all probability to be found in the practice of retaining captured fishes in pens for the purpose of permitting eggs and sperms to mature.

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[3] "Presented at the meeting by Herschel Whitaker and erroneously attributed to him by the editor of the Transactions." (Quotation from Dean's Bibliography of Fishes, Vol. II, p. 329, published by the American Museum of Natural History, New York, 1917.)